Remarks

Support for the Amendments

Support for the amendments to claims 35, 57, 58, 78, 96, 97, 115, 132, 133, 149, 151, 159, 187, 196, 197 and 213 and for new claims 226 and 227 can be found throughout the specification. Specifically, support for the amendments to claims 35 and 78 can be found, *inter alia*, at page 14, lines 21-23. Support for the amendments to claims 57, 58, 96, 97, 132, 133, 159, 196 and 197 can be found, *inter alia*, at page 8, lines 11-20; at page 16, lines 26-31; throughout pages 31-33; and throughout the Examples, particularly, Examples 1, 2 and 5. Support for the amendments to claims 115, 151, 159, 187 and 213 can be found at page 14, lines 21-23; at page 16, lines 22-31; at page 18, lines 24-29; at page 19, lines 9-24; and throughout Example 6, particularly throughout pages 50 and 51. Support for the amendment to claim 149 can be found at page 8, lines 11-20 and throughout Example 6. Support for new claims 226 and 227 can be found at page 7, lines 25-27; at page 32, line 26 through page 33, line2; and in Figure 3D. Therefore, these amendments do not add new matter, and their entry and consideration are respectfully requested.

Status of the Claims

By the foregoing amendments, claims 35, 57, 58, 78, 96, 97, 115, 132, 133, 149, 151, 159, 187, 196, 197 and 213 are sought to be amended and new claims 226 and 227 are sought to be added. Upon entry of the foregoing amendments, claims 35-227 are pending in the application, with claims 35, 78, 115, 151, 159, 187 and 213 being the independent claims.

Summary of the Office Action

In the Office Action dated March 23, 2004, the Examiner has made seven rejections of the claims. Based on the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

The Rejection Under 35 U.S.C. § 102(b) Over Johnson

In the Office Action at pages 3-5, the Examiner has rejected claims 35-71, 74-77, 115-142, 145-180, 183-204, 207-220 and 223 under 35 U.S.C. § 102(b), as being anticipated by Johnson *et al.* WO 93/19172 (hereinafter "Johnson"). Applicants respectfully traverse this rejection.

The Rejection of Claims 115-142, 145-157 and 220

Present claim 115 (and hence claims 116-142 and 145-150 that depend ultimately therefrom and that are also rejected over Johnson) recites a method of producing a nucleic acid molecule, comprising: providing a first nucleic acid molecule comprising at least one promoter and at least a first recombination site; providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or portion thereof and at least a second recombination site; and forming a mixture between the first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between the first and second recombination sites, thereby producing a third nucleic acid molecule in which the promoter and the antibiotic resistance gene or portion

thereof are operably linked to form a functional antibiotic resistance gene, wherein the at least one recombination protein is not a transposase.

Present claim 151 (and hence claims 152-157 and 220 that depend ultimately therefrom and that are also rejected over Johnson) claims a method of producing a nucleic acid molecule comprising: providing a first nucleic acid molecule comprising at least one promoter and at least a first *lox*P site; providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or portion thereof and at least a second *lox*P site; and forming a mixture between the first and second nucleic acid molecules and at least one Cre recombination protein, under conditions sufficient to cause recombination between the first and second *lox*P sites, thereby producing a third nucleic acid molecule in which the promoter and the antibiotic resistance gene or portion thereof are operably linked to form a functional antibiotic resistance gene.

As noted in Applicants' response of December 16, 2002 (incorporated by reference herein in its entirety), and as the Examiner acknowledges in the present Office Action (*see* page 3, third paragraph), Johnson discloses methods for the "shuffling" of heavy (V_H) chains of an antibody molecule by recombination of two nucleic acid molecules, one of which encodes a first V_H antibody chain and the other of which encodes a second, different V_H antibody chain. Johnson, however, provides no disclosure of the operable linkage of a promoter on a first nucleic acid molecule with an antibiotic resistance gene or portion thereof on a second nucleic acid molecule, as recited in present claims 115 and 151.

Under 35 U.S.C. § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference. *See Kalman v.*

Kimberly Clark Corp., 713 F.2d 760, 711 (Fed.Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984). Applicants respectfully submit that Johnson does not disclose the presently claimed invention. Hence, in view of *Kalman*, Johnson does not anticipate claims 115-142, 145-157 and 220. Reconsideration and withdrawal of this portion of the rejection therefore are respectfully requested.

The Rejection of Claims 35-71, 74-77, 158-180 and 183-186

Present claim 35 (and hence claims 36-71, 74-77 and 158 that depend ultimately therefrom and that are also rejected over Johnson) recites a method of producing a nucleic acid molecule comprising: providing a first nucleic acid molecule comprising at least a first gene or portion thereof and at least a first recombination site; providing a second nucleic acid molecule comprising at least a second gene or portion thereof and at least a second recombination site; and forming a mixture *in vitro* between the first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination *in vitro* between the first and second recombination sites, thereby producing a third nucleic acid molecule in which the first and second genes or portions thereof are operably linked to form a functional gene, wherein the at least one recombination protein is not a transposase.

Present claim 159 (and hence claims 160-180 and 183-186 that depend ultimately therefrom and that are also rejected over Johnson) recites a method of producing a nucleic acid molecule comprising: providing a first nucleic acid molecule comprising at least one promoter located immediately adjacent to at least a first recombination site; providing a

second nucleic acid molecule comprising at least one gene or portion thereof located immediately adjacent to at least a second recombination site; and forming a mixture *in vitro* between the first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination *in vitro* between the first and second recombination sites, thereby producing a third nucleic acid molecule in which the at least one promoter and the at least one gene or portion thereof are operably linked to form a functional gene, wherein the at least one recombination protein is not a transposase.

Present claims 35 and 159 are drawn to methods in which the recombination reaction takes place *in vitro*, *i.e.* outside of host cells. The Examiner contends that Johnson discloses that recombination reactions may be performed *in vitro*. Applicants respectfully disagree with this contention, and note that Johnson only mentions *in vitro* Cre-catalyzed recombination in passing (*see, e.g.*, Johnson at page 21, lines 9-11). Applicants incorporate by reference herein their previous remarks made in their reply of January 5, 2004. In addition, Applicants offer the following additional remarks to overcome this rejection.

The Examiner contends that the difficulties of performing *in vitro* site-specific recombination are unsupported by the instant application and the related art. The Examiner further states "[i]t would have only required routine experimentation to determine the optimal reaction conditions in order to practice the methods of Johnson *in vitro*." Office Action, at page 5, lines 2-4. Applicants respectfully disagree with these contentions.

The Examiner's assertion that the difficulties of performing *in vitro* site-specific recombination are unsupported by the instant application and the related art is unsubstantiated. The Examiner's attention is directed to the present specification where

some of the difficulties with performing *in vitro* recombination are specifically discussed, outlining the need for the present invention:

> Although site specific recombinases have been used to recombine DNA *in vivo*, the successful use of such enzymes *in vitro* was expected to suffer from several problems. For example, the site specificities and efficiencies were expected to differ *in vitro*; topologically-linked products were expected; and the topology of the DNA substrates and recombination proteins was expected to differ significantly *in vitro* (*see, e.g.*, Adams *et al.*, *J. Mol. Biol. 226*:661-73 (1992)). Reactions that could go on for many hours *in vivo* were expected to occur in significantly less time *in vitro* before the enzymes became inactive. Multiple DNA recombination products were expected in the biological host used, resulting in unsatisfactory reliability, specificity or efficiency of subcloning. *In vitro* recombination reactions were not expected to be sufficiently efficient to yield the desired levels of product.

Present specification at page 6, lines 1-16. Applicants respectfully submit that the instant application clearly supports and outlines the difficulties in performing *in vitro* recombination prior to the present invention. Applicants also submit that, while the related art acknowledges these difficulties, it is silent with respect to the buffers, reaction conditions and other enabling methods and experimentation that would allow the ordinarily skilled artisan to practice *in vitro* recombination. Finally, the Examiner has not pointed to any relevant art that would provide the requisite guidance to allow the ordinarily skilled artisan to practice the methods of Johnson *in vitro*.

With regard to the Examiner's assertion that it would have required only routine experimentation to practice the methods of Johnson *in vitro*, Applicants respectfully submit that the ordinarily skilled artisan would in fact have had to undertake extensive, undue experimentation in order to practice these methods *in vitro*. As set forth in *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), such undue experimentation precludes enablement

under 35 U.S.C. § 112, first paragraph. While some experimentation does not necessarily preclude enablement, Applicants submit that a significant amount of undue experimentation would be required in order for one of ordinary skill to have adapted the methods disclosed in Johnson to perform *in vitro* recombination. (*See PPG Indus., Inc. v. Guardian Indus. Corp.*, 37 USPQ2d 1618, 1623 (Fed. Cir. 1996), *citing Atlas Powder Co. v. E.I. DuPont De Nemours & Co.*, 224 USPQ 409, 413 (Fed. Cir. 1984)). Johnson provides no experimental details in support of *in vitro* recombination, as all of the protocols in the Examples in Johnson are limited to *in vivo* recombination wherein the recombination takes place inside of host cells (*see, e.g.,* Johnson in Example 1, at page 45, and in Example 2, at page 51). Hence, Johnson supplies no direction or guidance for the experimentation required by the Examiner, and furthermore provides no working examples of *in vitro* recombination that would aid the ordinarily skilled artisan in such experimentation. While the level of the skill of those in the art is fairly high, the cited art provides no guidance needed by one of ordinary skill in order to practice the methods of Johnson *in vitro*. Therefore, at best, one of ordinary skill would have to undertake undue experimentation to be able to use the methods in Johnson to perform *in vitro* recombination. Hence, Applicants submit that for at least these reasons, Johnson does not enable *in vitro* recombination.

As the Federal Circuit has held, a claim can only be anticipated by a publication if the publication describes the claimed invention with sufficient enabling detail to place the public in possession of the invention. See *In re Donohue*, 766 F.2d 531, 533 (Fed. Cir. 1985); *see also PPG Industries, Inc.,* 75 F.3d at 1566 ("To anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to

make the anticipating subject matter."). For at least the reasons discussed above, Applicants respectfully submit that Johnson does not provide an enabling disclosure of the present invention. Hence, in view of *Donohue* and *PPG Industries*, Johnson does not anticipate the presently claimed invention.

In view of the foregoing remarks, Applicants respectfully assert that Johnson is a deficient reference, and cannot be relied upon to reject the presently claimed invention under 35 U.S.C. § 102(b). Reconsideration and withdrawal of the rejection of claims 35-71, 74-77, 115-142, 145-180, 183-204, 207-220 and 223 under 35 U.S.C. § 102(b) over Johnson therefore are respectfully requested.

The Rejection of Claims 187-204, 207-219 and 223

Present claim 187 (and hence claims 188-204 and 207-212 that depend ultimately therefrom and that are also rejected over Johnson) recites a method of producing a nucleic acid molecule comprising: providing a first nucleic acid molecule comprising at least one promoter and at least a first recombination site; providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or portion thereof and at least a second recombination site; and forming a mixture *in vitro* between said first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination *in vitro* between said first and second recombination sites, thereby producing a third nucleic acid molecule in which the promoter and the antibiotic resistance gene or portion thereof are operably linked to form a functional antibiotic resistance gene, wherein the at least one recombination protein is not a transposase.

Present claim 213 (and hence claims 214-219 and 223 that depend ultimately therefrom and that are also rejected over Johnson) recites a method of producing a nucleic acid molecule comprising: providing a first nucleic acid molecule comprising at least one promoter and at least a first *lox*P site; providing a second nucleic acid molecule comprising at least one antibiotic resistance gene or portion thereof and at least a second *lox*P site; and forming a mixture *in vitro* between the first and second nucleic acid molecules and at least one Cre recombination protein, under conditions sufficient to cause recombination *in vitro* between the first and second *lox*P sites, thereby producing a third nucleic acid molecule in which the promoter and the antibiotic resistance gene or portion thereof are operably linked to form a functional antibiotic resistance gene. Present claims 187 and 213 provide for the *in vitro* recombination of a first and second nucleic acid molecule to produce a third nucleic acid molecule in which a promoter and an antibiotic resistance gene or portion thereof are operably linked to form a functional antibiotic resistance gene.

As noted above, Johnson does not disclose the operable linkage of a promoter on a first nucleic acid molecule with an antibiotic resistance gene or portion thereof on a second nucleic acid molecule. Furthermore, Johnson does not enable *in vitro* recombination for the reasons discussed above. Hence, Johnson does not disclose every element of the presently claimed invention.

In view of the foregoing remarks, reconsideration and withdrawal of the rejection of claims 187-204, 207-219 and 223 under 35 U.S.C. § 102(b) over Johnson are respectfully requested.

Summary

In view of the foregoing remarks, Applicants respectfully assert that claims 35-71, 74-77, 115-142, 145-180, 183-204, 207-220 and 223 are not anticipated by Johnson. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 102(b) over Johnson therefore are respectfully requested.

The Rejection Under 35 U.S.C. § 102(e) Over Demirjian

In the Office Action at pages 5-7, the Examiner has rejected claims 35-42, 49, 57-59, 66-71, 74-81, 88, 96-98, 102-106, 109-117, 124, 132-134, 138-142, 145-150, 159-163, 176-180, 183-189, 196-197, 201-204 and 207-212 under 35 U.S.C. § 102(e), as being anticipated by Demirjian *et al.*, U.S. Patent No. 5,981,177 (hereinafter "Demirjian"). Applicants respectfully traverse this rejection.

The Examiner contends that Demirjian discloses mixing a first and second nucleic acid with a recombination protein to recombine the first and second nucleic acids to form a third nucleic acid, thereby forming an operably liked, functional gene from the first and second portions of the gene. The Examiner indicates that the first and second portions of the gene may be fragments of the gene, may comprise a promoter and may encode a selectable antibiotic marker. The Examiner further asserts that the transposition disclosed in Demirjian was necessarily mediated by some sort of recombination protein, whether or not it was a transposase, and therefore concludes that Demirjian discloses the presently claimed invention. Applicants respectfully disagree with this conclusion.

Present claim 78 (and hence claims 79-81, 88, 96-98, 102-106 and 109-114 that depend ultimately therefrom and that are also rejected over Demirjian) recites a method of producing a nucleic acid molecule, comprising: providing a first nucleic acid molecule comprising a first portion of an antibiotic resistance gene and at least a first recombination site; providing a second nucleic acid molecule comprising a second portion of the antibiotic resistance gene and at least a second recombination site; and forming a mixture between the first and second nucleic acid molecules and at least one recombination protein, under conditions sufficient to cause recombination between the first and second recombination sites, thereby producing a third nucleic acid molecule in which the first and second portions of the gene are operably linked to form a functional antibiotic resistance gene, wherein said at least one recombination protein is not a transposase.

Present independent claims 35 (and hence claims 36-42, 49, 57-59, 66-71 and 74-77 that depend ultimately therefrom and that are also rejected under Demirjian), 78 (and hence claims 79-81, 88, 96-98, 102-106 and 109-114 that depend ultimately therefrom and that are also rejected under Demirjian), 115 (and hence claims 116-117, 124, 132-134, 138-142 and 145-150 that depend ultimately therefrom and that are also rejected under Demirjian), 159 (and hence claims 160-163, 176-180 and 183-186 that depend ultimately therefrom and that are also rejected under Demirjian) and 187 (and hence claims 188-189, 196-197, 201-204 and 207-212 that depend ultimately therefrom and that are also rejected under Demirjian) all recite methods of producing nucleic acid molecules that comprise forming a mixture between a first and second nucleic acid molecules and at least one recombination protein that is not a transposase. Applicants respectfully submit that Demirjian does not disclose the

presently claimed invention, as the methods of Demirjian require the use of a transposase in conjunction with the Mu transposon. (*See* Demirjian at column 2, lines 28-39; and at column 24, lines 6-9). Therefore, Demirjian does not disclose every element of the present invention, and in view of *Kalman*, Demirjian cannot and does not anticipate the present invention.

In view of the foregoing remarks, reconsideration and withdrawal of the rejection of claims 35-42, 49, 57-59, 66-71, 74-81, 88, 96-98, 102-106, 109-117, 124, 132-134, 138-142, 145-150, 159-163, 176-180, 183-189, 196-197, 201-204 and 207-212 under 35 U.S.C. § 102(e) over Demirjian are respectfully requested.

The Rejections Under 35 U.S.C. § 112, Second Paragraph

In the Office Action at pages 7-9, the Examiner has rejected claims 33-225 under 35 U.S.C. § 112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants respectfully traverse these rejections as follows.

The Examiner has first rejected claims 33-225 alleging that the phrase "portions thereof" as it relates to portions of genes is unclear. Applicants respectfully traverse this rejection. In view of the foregoing amendments, each of the independent claims of the present invention recite "or portion therefore are operably linked to form a functional (antibiotic resistance) gene." Applicants respectfully submit that the ordinarily skilled artisan would readily understand that the term "portion" of a gene as used in the present invention would encompass any fragment or part of a gene, including a single nucleotide, so

long as when it was operably linked using the methods of the present invention to another portion of a gene (or promoter) it formed a functional gene (or functional antibiotic resistance gene). The Examiner has noted that the rejection under 35 U.S.C. § 102(b), over Johnson, was not applied to claim 78 due to the limitation "to form a functional antibiotic resistance gene." *See* Office Action at page 5, lines 14-16. Applicants respectfully submit that inclusion of similar language in all of the independent claims clearly points out and distinctly claims the subject matter which Applicants regard as the invention. Hence, Applicants respectfully request reconsideration and withdrawal of this rejection under 35 U.S.C. § 112, second paragraph.

The Examiner has next rejected claims 57-58, 96-97, 132-133 and 196-197 under 35 U.S.C. § 112, second paragraph, alleging that the term "adjacent" is unclear. Applicants respectfully traverse this rejection.

Present claims 57-58, 96-97, 132-133 and 196-197 recite the term "immediately adjacent" with respect to the structural relationship between the recited recombination site(s) and the related genes, portions of genes, antibiotic resistance genes, portions of antibiotic resistance genes or promoters, as recited in the various claims. Applicants respectfully submit that the ordinarily skilled artisan would readily understand, as the Examiner has correctly indicated (*see* Office Action at page 8, lines 11-13), that the term "immediately adjacent" means that the recombination sites and the related genes, portions of gene, etc., have no intervening nucleotides between them. As such, Applicants submit that the term "immediately adjacent" is neither vague nor indefinite. In view of the foregoing remarks,

Applicants respectfully request that the rejection of claims 57-58, 96-97, 132-133 and 196-197 under 35 U.S.C. § 112, second paragraph, be reconsidered and withdrawn.

The Examiner has next rejected claims 98 and 134 under 35 U.S.C. § 112, second paragraph, alleging that the term "at least one cloning site" is not explicitly defined in the specification, and this it is unclear what structural or functional characteristics are intended by the phrase "cloning site." Applicants respectfully traverse this rejection. Applicants respectfully submit that the ordinarily skilled artisan would readily understand that the term "cloning site" encompasses any site within a given DNA molecule which allows for insertion of a desired sequence. The Examiner's attention is drawn to the present specification at page 32, line 27, through page 33, line 2, where the vector pEZC602 shown in Figure 3D is described. The vector comprises "*lox*P and *lox*P511 sites flanking a multiple cloning site." Figure 3D clearly shows the position of the *lox*P and *lox*P511 sites and the multiple cloning site region between them. Applicants submit that the term "cloning site" is therefore sufficiently defined in the present specification and would be readily understood by the ordinarily skilled artisan. In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 98 and 134 under 35 U.S.C. § 112, second paragraph.

The Examiner has also rejected claim 149 under 35 U.S.C. § 112, second paragraph, alleging that there is no clear and positive antecedent basis for the term "said nucleic acid" in claim 115, upon which claim 149 depends. By the foregoing amendments, claim 149 has been amended to recite "said third nucleic acid," and thus this rejection has been fully

accommodated. Therefore, Applicants respectfully request reconsideration with withdrawal of this rejection.

Finally, the Examiner has rejected claim 158 under 35 U.S.C. § 112, second paragraph, alleging that the phrase "wherein said first gene or portion thereof, and said second gene or portion thereof are the same," is vague and indefinite. Applicants respectfully traverse this rejection. Applicants submit that the ordinarily skilled artisan would understand the phrase "wherein said first gene or portion thereof, and said second gene or portion thereof, are the same," to mean that, as stated, the first and second gene or portions thereof are the *same* structurally and functionally, in all respects. The ordinarily skilled artisan would recognize that utilizing such an embodiment of the present invention would allow for the production of, for example, a multiple copy vector, or simply a nucleic acid molecule comprising two copies of the same gene. Applicants respectfully submit that this phrase is neither vague nor indefinite, and would be clearly understood by the ordinarily skilled artisan. In view of the foregoing remarks, Applicants respectfully request that the rejection of claim 158 under 35 U.S.C. § 112, second paragraph, be reconsidered and withdrawn.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated or otherwise overcome. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn.

Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

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